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Common house spiders could be vectors of Community-Acquired Methicillin-Resistant *Staphylococcus aureus*.

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Abstract

Aim: To identify whether common household spiders such as *Tegenaria saeva* and *Amaurobius ferox* are likely vectors and reservoirs for Methicillin-Resistant *Staphylococcus aureus*.

Methods and Results: Spiders were tested for the presence of *S.aureus* on the fangs, lower and upper abdomen and the legs. *S.aureus* was isolated from the fangs and isolates were subcultured to test for oxacillin resistance. Of the sample that was tested a mean of 83.4 x 10^2 cfu/fang for total counts of *S.aureus* was found. While 23% of the isolates tested for oxacillin-resistance were oxacillin resistant.

Conclusion: Common household spiders can easily act as vectors and a community reservoir for methicillin-resistant *Staphylococcus aureus*.

Significance and Impact of Study: As *T.savea* and *A.ferox* have been identified as possible vectors and reservoirs of MRSA; higher levels of control need to be implemented in the monitoring of household spiders in their movements as well as colonization rates of *S.aureus*. Classifications of MRSA strains is based upon the Staphylococcal Cassette Chromosome *mec* (SCCmec) that the strain carries, however some genetic analyses aren't able to fully distinguish between SCCmec types and the subtypes due to the complexity of the SCCmec. Thus further analysis of the SCCmec and strains of MRSA needs to be undertaken. This is essential for the control and prevention of the spread of MRSA throughout our community.

Keywords:

Methicillin-Resistant *Staphylococcus aureus* • *SCCmec* • Community- Hospital-Acquired • *Tegenaria saeva* • *Amaurobius ferox*

Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is now becoming a world-wide problem as the bacterium is now no longer confined to the hospital setting and being isolated in individuals with no established risk factors (Klutymans-Vandenbergh *et al.* 2006). This has lead to an increased need to identify potential reservoirs and vectors for MRSA in the community. The large majority of CA-MRSA strains found in the community are thought to belong to five distinct clonal lineages (Diep and Otto 2008). It is known that these five strains have specialised pathogenic traits that HA-MRSA strains do not carry (Diep and Otto 2008). The ability to distinguish between Hospital-Acquired MRSA (HA-MRSA) and Community-Acquired MRSA (CA-MRSA) is needed for control and prevention of the spread of MRSA in the community. Epidemiological definitions are usually based on the timing of colonization in relation to the patient being admitted to the hospital setting (Klutymans-VandenBergh *et al.* 2006). But problems with these definitions have been revealed because both methicillin-susceptible and methicillin-resistant *S.aureus* strains can be asymptomatic from anything between months and years. This consequently leads to an over- or even an under-estimation of the true prevalence of either CA-MRSA or HA-MRSA. As a result of this, classification of MRSA strains is now determined by the Staphylococcal Cassette Chromosome *mec* (SCCmec) type, the presence of additional antibiotic resistance genes, bacterial growth and the distribution of toxin genes. It is known that CA-MRSA strains usually carry SCCmec type IV or V (Okuma *et al.* 2002) while the majority of HA-MRSA strains contain SCCmec types I, II or III (Ito *et al.* 2001). CA-MRSA antibiotic resistance is usually confined to beta-lactam antibiotics (Gorak *et al*. 1999) while HA-MRSA is known to have multi drug resistance (Klutymans-VandenBergh *et al*. 2006). This appears to be consistent with the absence of antibiotic resistance genes other than the mecA in SCCmec types IV and V, when compared to the accumulation of multiple additional resistance genes in SCCmec types II and III (Ito *et al*. 2001, Ma *et al.* 2002).

In many cases in the USA, where MRSA has caused skin and soft tissue infections it is often perceived as being preceded by a spider bite. There are several explanations to this; firstly being that spiders introduce MRSA into a bite wound and thus act as vector for the spread of MRSA, secondly MRSA colonization is a secondary event to a spider bite and thirdly spider bites are a misguided way for patients to explain why they have skin and soft tissue infections (Baxtrom *et al*. 2006). With this in mind it is plausible to believe that if common house hold spiders do act as a vector for MRSA then they should be consequently colonized by MRSA**.**

The aim of this study was to sample household spiders such as *Tegenaria saeva* and *Amaurobius ferox* and screen them for *S.aureus* as well as fungal species and *Enterococcus* spp. Then *S.aureus* isolates were tested for oxacillin resistance. This was to identify whether these household spiders are a likely vector and reservoir for MRSA.

Methods and Materials

Spider collection. Fellow students of the University of Plymouth were asked to collect any spiders that were found within a household environment along with a description and a picture of the

Tegenaria saeva and *Amaurobius ferox*. The collected spiders were identified using Roberts (2009). Spiders were contained in an aseptic environment; until there was space and time to process them. To test the external microbial flora on the spiders, swabs were taken of the upper and lower abdomen and a swab of two legs per spider were taken. To test the internal flora of the spiders, the fangs of each spider were dissected and put into 5ml of phosphate buffer saline pH 7.0 (PBS).

Microbial culture. Aseptic technique was used throughout the experiments carried out. Media were made up to select for certain bacterial and fungal species. Slanetz and Barley (S&B Oxoid) was made up to select for any *Enterococcus* spp. While Rose-Bengal Chloramphenicol Agar (RBCA Oxoid) was made up to select for fungi present on the spiders fangs. Tryptone Soy Agar (TSA Oxoid) was made up for total bacteria count. To select for *Staphylococcus aureus,* Staphylococcus medium 110 (Staph110 Oxoid) was used. To test for oxacillin-resistance Mueller-Hinton agar (Lab M) with 4% NaCl embedded with oxacillin at a concentration of 8mg/l was made up and isolates were taken from the Staph110 plates and sub-cultured onto the Mullen-Hinton plates.

Spider processing. 1ml of the PBS fang diluent was then spread plated on to Staph110, RBCA, S&B and TSA; then both RBCA and TSA plates were incubated at 25^0C for 48 hours. The S&B and Staph110 plates were incubated at 35^0C for 48 hours. Once *S.aureus* had been isolated on the Staph110 plates a single colony was streak plated on to the Mueller-Hinton agar and then incubated for 48 hours at 35^0C. Each Mueller-Hinton agar plate was split into 5 sections to enable each plate to have multiple colonies from each particular spider to be tested at once. A positive control was carried out for both the Staph110 plates and S&B plates to show that the plates were able to select for both *S.aureus* and *Enterococcus* spp. A single Muellen-Hinton plate was divided into two sections and inoculated with an MRSA strain and a methicillin-susceptible *Staphylococcus aureus* (MSSA), to identify whether the oxacillin had successfully been taken up by the Muellen-Hinton agar. Total counts were taken for all Staph110 plates and TSA to enable a quantification of the spread of *S.aureus* and the diversity of the microbial flora present on the fangs.

Results

Table 1 shows that only two swabs (spiders 8 and 10), both from the lower abdomen, resulted in growth of *S.aureus* when plated on to the Staph110 medium. All other swabs came back without any growth when plated onto the Staph110 medium. All swabs were aseptic and brushed twice over the area of spider that was being sampled. Once the swabs were taken the fangs were then removed and left suspended in PBS for 48 hours.

After this period of 48 hours a known volume of the PBS fang diluent was pipetted onto a TSA, RBCA, Staph110 and S&B plate for each spider. The suspension was then spread plated (to ensure universal distribution of the diluent) and incubated for 48 hours at suitable temperatures for each plate. After the period of 48 hours the plates were then studied to see whether there had been growth on any of the plates.

Table 1. Swabs showing presence or no presence of *S.aureus* on Lower and Upper Abdomen and Legs of *T.savea* and *A.ferox*

	Upper Abdomen	Lower Abdomen	Legs	
Spider 1 (*T.savea*)	-	-	-	+=presence
Spider 2 (*T.savea*)	-	-	-	-=no presence
Spider 3 (*T.savea*)	-	-	-	
Spider 4 (*T.savea*)	-	-	-	
Spider 5 (*T.savea*)	-	-	-	
Spider 6 (*T.savea*)	-	-	-	
Spider 7 (*T.savea*)	-	-	-	
Spider 8 (*T.savea*)	-	+	-	
Spider 9 (*A.ferox*)	-	-	-	
Spider 10 (*A.ferox*)	-	+	-	
Spider 11 (*A.ferox*)	-	-	-	

Table 2. Internal Microbial Flora from fangs of Spiders

	TSA	Staph110	RBCA	S&B	
Spider 1 (*T.savea*)	+	+	-	-	+= growth
Spider 2 (*T.savea*)	+	+	+	-	-=no growth
Spider 3 (*T.savea*)	+	-	+	-	
Spider 4 (*T.savea*)	+	+	+	-	
Spider 5 (*T.savea*)	+	-	-	-	
Spider 6 (*T.savea*)	+	+	+	-	
Spider 7 (*T.savea*)	+	+	+	-	
Spider 8 (*T.savea*)	+	+	+	-	
Spider 9 (*A.ferox*)	+	+	+	-	
Spider 10 (*A.ferox*)	+	+	+	-	
Spider 11 (*A.ferox*)	+	-	+	-	

Table 2 shows that all the spiders sampled came back with no growth on the S&B plates showing that there were no *Enterococcus* spp. present. It was seen that for every spider there was growth on the TSA plates. These were meant to be accompanied by a total count for each TSA plate but as there was so much growth total counts were unable to be performed. All the RBCA plates came back with growth apart from spiders 1 and 5; all plates were again fully covered with growth so this made a total count impossible by eye. But this leads to thoughts that the microbial flora is incredibly diverse within the spiders' fangs.

For Staph110 plates (Table 3) 73% came back positive for *S.aureus* growth, only spiders 3, 5 and 11 had no growth on the Staph110 plates. Total counts were done for each spider that came back positive for growth and these results a had mean of 83.4 $x10^2$ cfu/fang and a standard deviation of 144.4 $x10^2$ cfu/fang. These two readings show the wide scope for the colonization of *S.aureus* within the spiders' fangs. The smallest total count being 0.125 $x10^2$ cfu/fang compared to the largest of 3900 $x10^2$ cfu/fang. It is also seen that both *T.savea* and *A.ferox* seem to be equally adept as a vector for *S.aureus*.

Table 3. Total Counts of *S.aureus* per fang from Staph110 plates			
	Total Count cfu/fang x10^2		
Spider 1 (*T.savea)*	265	Standard Deviation =	144.4 x 10^2
Spider 2 (*T.savea)*	1.25	Mean=	83.4 x 10^2
Spider 4 (*T.savea)*	7.23		
Spider 6 (*T.savea)*	2.81		
Spider 7 (*T.savea)*	0.125		
Spider 8 (*T.savea)*	0.25		
Spider 9 (*A.ferox)*	0.5		
Spider 10 (*A.ferox)*	3900		

Finally isolates from the Staph110 plates were then inoculated and streaked onto the Muellen-Hinton plates for oxacillin-resistance testing. There were 57 *S.aureus* isolates plated onto Muellen-Hinton plates. Each Muellen-Hinton plate was divided into sections to allow multiple isolates from one spider to be tested.

Table 4 shows the isolates that were tested for oxacillin-resistance, with 23% of all isolates tested being positive for oxacillin-resistance. While there was varying degrees of resistance within each spider, for example all of the isolates from spider 2 were oxacillin-resistant, while spiders 7, 8 and 10 showed that no isolates tested were oxacillin-resistant

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Table 4. *S.aureus* isolates tested for oxacillin-resistance		
	No. of *S.aureus* isolates plated	No. of isolates that were oxacillin-resistant
Spider 1 (*T.savea)*	10	4
Spider 2 (*T.savea)*	5	5
Spider 4 (*T.savea)*	10	1
Spider 6 (*T.savea)*	8	1
Spider 7 (*T.savea)*	4	0
Spider 8 (*T.savea)*	4	0
Spider 9 (*A.ferox)*	8	2
Spider 10 (*A.ferox)*	8	0
Total isolates with oxacillin-resistance= 23%		

Discussion

Classification of MRSA strains was traditionally done by the timing of colonization in relation to admittance to a hospital setting (Klutymans-Vandenbergh *et al.* 2006), but this style of classifying the strains of MRSA strain leads to over and under estimation of true prevalence of CA-MRSA and HA-MRSA. Because of this molecular epidemiological data was researched to see if more reliable definitions could be made.

Within this study it has been seen that common house spiders could easily be a vector for MRSA, oxacillin-resistance was tested (which is a β-lactam and according to Gorak *et al.* 1999 this means that the strain can be classified as a CA-MRSA strain) for *S.aurues* colonies isolated from the spiders. While HA-MRSA are traditionally classified as HA-MRSA because they have multi drug resistance (Klutymans-Vandenbergh *et al*. 2006). This means that further antibiotic testing would be needed for classification of the *S.aureus* isolates found. This could be done by testing the *S.aureus* isolates against tetracylines such as minocycline and doxycycline, which HA-MRSA strains are thought to be resistant to (Naimi *et al*. 2003) where CA-MRSA strains are not. The antibiotic resistance profiles of both forms of MRSA strains are due to antibiotic resistance genes such as mecA in SCCmec types IV and V, compared to the gathering of multiple antibiotic resistance genes in SCCmec types II and III (Okuma *et al.* 2002).

Classification of MRSA strains is not however based upon the antibiotic resistance profiles, but it would lead to larger understanding of the strain and whether it could possibly be a HA-MRSA or a CA-MRSA strain. This indecisiveness, of which strains can be classified as CA-MRSA strains, arises from the extreme heterogeneity of the genetic background of *S.aureus*. This has lead to belief that any MRSA strain has the potential to become a CA-MRSA strain (Harbarth *et al*. 2005 and Fang *et al.* 2008).

Classification of MRSA strains is currently done by SCCmec typing into either SCCmec types I, II, III, IV, or V. The SCCmec consists of two essential genetic elements, being the mec gene complex and the ccr gene complex and the junkyard (J) region (Ito *et al*. 2001, Ma *et al.* 2002 and Ito et al 2004). There are three classes of mec complex (A, B and C) along with four allotypes of ccr complex (1, 2, 3 and 4). Different combinations of these complexes give rise to the different SCCmec types and they are further subtyped according to differences in the J region (Ito *et al*. 2001, Ma *et al*. 2002 and Ito *et al.* 2004). It's known that most CA-MRSA strains carry SCCmec types IV or V (Okuma *et al*. 2002) while HA-MRSA has types I, II or III (Oliveria and de Lencastre 2002). Both SCCmec types IV and V are smaller in size than types I, II and III which gives these types more mobility through horizontal gene transfer (Okuma *et al.* 2002). As well as this it is reported that CA-MRSA strains that carry SCCmec type IV have an increased growth rate than other strains with varying SCCmec types (Okuma *et al*. 2002), this could be an explanation for the wide scope of colonization within the sample, and thus rising questions of which strain of MRSA the spiders maybe colonized with. This is important to distinguish as both *T.savea* and *A.ferox* are frequently in contact with the community. It is also important to recognise that the *T.savea* and *A.ferox* may come into contact with each other, spreading MRSA strains further into the spider community and consequently ours.

Because of the complexity and the structural diversity of the SCCmec types, classifying them into correct categories is done by either DNA sequence analysis (Ito *et al.* 1999, Ito *et al*. 2001 and Oliveira *et al*. 2001) or by Southern Blot analysis by using three or more restriction enzymes as well as several key probes specific for each concerning SCCmec type (Oliveira *et al*. 2001) and by PCR. Previously described PCR SCCmec

typing programmes were designed to target individual regions of the classes of the *mec* complex, allotypes of the *ccr* complex and individual subtypes of the J regions; thus the method requires many sets of primers and PCR experiments (Okuma *et al*. 2002 and Ito *et al*. 2004). These methods devised by Okuma *et al*. 2002 and Ito *et al*. 2004 are time-consuming, laborious and expensive; resulting in less accessibility for clinical and surveillance processes. Oliveira and de Lencastre 2002 developed a multiplex PCR system for *mec* element type assignment and defined type of SCCmec according to the genes location within the J regions of the SCCmec elements. Along with Zhang *et al*. 2005, Oliveira and de Lencastre 2002 systems are the only single-step multiplex PCR assays published to date which are simpler and easier to perform than non-multiplex PCR assays. However Zhang *et al*. 2005 did discover limitations within Oliveira and de Lencastre 2002 in terms of detecting newly described SCCmec type V and misclassifying them as SCCmec type III, while also failing to distinguish between SCCmec type IV into appropriate subtypes a, b, c and d (Oliveira and de Lencastre 2002). Therefore detecting SCCmec types V and discriminating SCCmec type IV into appropriate subtypes play an important role in fully understanding the epidemiology and ultimately the control and prevention of emerging CA-MRSA outbreaks (Zhang *et al.* 2005).

Thus the need for further analysis of the *S.aureus* isolates from the common household spiders needs to be extended, with SCCmec typing essential for control and prevention of the spread of these strains found on the spiders. Once SCCmec typing has been done then antibiotic resistance testing can be used to further support the classification of which CA-MRSA strain was found within the spiders' microbial flora. It will be necessary also to monitor the horizontal genetic transfer between MRSA strains. As it has been seen the strains are able to pass components of the SCCmec types and thus creating new SCCmec types.

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